Journal of Medical Virology 59:194–197 (1999)

Is Sexual Transmission an Important Pattern for Herpes Simplex Type 2 Virus Seroconversion in the Spanish General Population?

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Herpes simplex type 2 (HSV-2) seroprevalence within a community is determined by sexual and perinatal transmission from mother to baby, the two main sources of virus shedding. A seroepidemiological study of HSV-2 was undertaken on a representative sample (n = 3974) of the Spanish population to assess indirectly the relative relevance of these two transmisssion routes. The sample comprised 1922 men and 2052 women in the age range 5–59 years, stratified by sex and age (5–12, 13–19, 20–29, 30–39, 40–49, and 50–59 years). Sera were screened for HSV-2 specific Ig G antibodies by an enzyme-linked immunoabsorbent assay based on recombinant glycoprotein G2 (gG2). The overall prevalence of antibodies to HSV-2 was 3.6% (95% CI: 3.1–4.2%). Prevalence by gender did not differ: males (3.6%; 95% CI: 2.8–4.6%) and females (3.6%; 95% CI: 2.8–4.5%). There were no significant differences between age groups with respect to seropositivity rates. Detection of HSV-2 antibodies was not associated with increasing age, as is expected for a sexually transmitted disease. The fact that seroprevalence rates among the different age groups did not differ suggests that the virus is not circulating in the general population and may be restricted to risk groups only. Similar positivity rates found in the group of females of childbearing age and in the youngest population indicate that perinatal viral shedding is the main source of HSV-2 seroconversion in the Spanish population. ***J. Med. Virol. 59:194–197, 1999.***

KEY WORDS: Herpes simplex type 2 virus; seroprevalence; epidemiology

INTRODUCTION

Herpes simplex type 2 (HSV-2) seroprevalence within a community is determined by sexual and perinatal transmission from mother to baby, the two main sources for virus shedding [Straus, 1995]. Serological procedures for HSV-2 typing and therefore seroepidemiological studies on HSV-2 have been traditionally limited by cross-reactivity to type common Herpes simplex type 1 antigens (HSV-1) [Corey and Spear, 1986; Mertz, 1993]. Few surveys describing the seroepidemiology of HSV-2 in Spain [Vizcaíno et al., 1987; Nahmias et al., 1990; De Sanjosé et al., 1994] and other European countries [Ades et al., 1989; Nahmias et al., 1990; Kjaer et al., 1990, 1993; Cowan et al., 1994; Forsgren et al., 1994] have been published. Most of such studies involved non representative samples of the general population and a variety of serological assays, thus providing controversial data which are difficult to interpret. At present, commercial availability of new serological methods based on type-specific proteins, such as glycoprotein G2 (gG2) may facilitate the performance of seroepidemiological studies that will provide more accurate estimates of the real prevalence of HSV-2 among different populations, as well as a better knowledge of the transmission patterns of the virus.

This study aimed at providing data on the seroprevalence of HSV-2 infection in a representative sample of the Spanish population in order to assess indirectly the relevance of HSV-2 sexual and perinatal transmission among the general population, using a type-specific ELISA based on recombinant gG2.

MATERIALS AND METHODS

Study Population and Design

The study was carried out on 3974 serum samples collected during 1992 and 1993 for the DRECE (Diet and Risk of Cardiovascular Disease in Spain) study,

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Accepted 16 March 1999

TABLE I. Prevalence of HSV-2 Antibodies According to Age and Sex

Age groups (years)	Overall				Males				Females			
	Total	n	%	CI	Total	n	%	CI	Total	n	%	CI
5–12	634	26	4.1	2.7–5.9	328	18	5.5	3.3–8.5	306	8	2.6	1.1–5.1
13–19	682	25	3.7	2.4–5.4	332	13	3.9	2.1–6.6	350	12	3.4	1.8–5.9
20–29	794	25	3.1	2.0–4.7	372	10	2.7	1.3–4.9	422	15	3.6	2.0–5.8
30–39	664	14	2.1	1.1–3.5	326	5	1.5	0.5–3.5	338	9	2.7	1.2–5.0
40–49	631	22	3.5	2.2–5.2	302	12	4.0	2.1–6.8	329	10	3.0	1.5–5.5
50–59	569	31	5.4	3.7–7.6	262	12	4.6	2.4–7.9	307	19	6.2	3.8–9.5
Total	3974	143	3.6	3.1–4.2	1922	70	3.6	2.8–4.6	2052	73	3.6	2.8–4.5

designed to find out the status of the Spanish population at risk of suffering cardiovascular disease [Gómez Gerique, 1997] and sponsored by the Spanish Ministry of Health. Sera were frozen and stored at −20°C until HSV-2 antibody testing was undertaken. The study protocol for this present seroepidemiological study was approved by the Research Ethics Committee of the University Clinic Hospital (Valencia, Spain).

The DRECE study was designed as a cross-sectional survey of the Spanish population aged 5 to 59 years and distributed in eight geographical regions. In each geographical area, the proportion of subjects included was correlated with the real proportion of subjects living in each region vs. the total Spanish population. Cluster sampling by sex, age, and geographical area was carried out. Each age stratum (5–12, 13–19, 20–29, 30–39, 40–49, and 50–59 years) comprised a number of subjects proportional to the real distribution in subjects of the Spanish population for each particular age range. The field work was undertaken in 53 randomly selected Primary Care Centres. Sample size for each center was calculated according to the population affiliated to each center. Subjects recruitment was by randomised selection from the census of potential public health users and when this was not possible, by a randomised routes system. Subjects with intercurrent diseases and those who had suffered any serious disease in the 3 months before enrolment were not included in the study.

Laboratory Tests

Type-specific Ig G antibodies to HSV-2 were detected by an indirect enzyme immunoassay (Captia Select HSV2-G, Centocor Inc., Malvern, NY). The test employs polystyrene microwells coated with recombinant type 2 antigen (modified gG-2) not shared by HSV-1. The recombinant antigen is produced in a baculovirus expression system, which minimises non-specific reactions and favours retention of the natural antigenic characters. In a previous study designed to assess the validity of the Captia Select HSV2-G assay, a pool of 159 serum samples known to be either anti HSV-2 positive or negative by a western blot technique (MRL Diagnostics, Cypress, California, USA), modified from Ashley et al. [1988] and tested at the MRL Reference Laboratory, were assayed by the recombinant enzyme immunoassay. Sensitivity and specificity of the test were found to be 95% and 92%, respectively [García-Corbeira et al., 1998].

Absorbances within 10% of the cut-off were considered equivocal results and were retested in duplicate to confirm their status. The final result was considered equivocal if absorbances were repeatedly within 10% of the cut-off. Equivocal samples were retested by a second enzyme immunoassay for detection of IgG antibodies against HSV-1 and HSV-2 (Herpesscan, Eurodiagnostica, Malmö, Sweden). The test employs microtitre wells coated with affinity purified gC (HSV-1), gG (HSV-2), or HSV common antigen. Both commercial kits were used according to the instructions of the manufacturers.

Sample Size Calculation and Statistical Analysis

From the total number of 4,787 healthy evaluable subjects (2,324 men and 2,463 women) that were finally included in the DRECE study, 3,974 sera (1,922 men and 2,052 women) were assayed for the present seroepidemiological study. This number was in accord with the volume of serum available for carrying out the serological tests. A recent Spanish study undertaken in women of childbearing age, involving a type-specific ELISA, showed an HSV-2 seroprevalence rate of 3.5% [Ory et al., 1997]. Assuming this rate as reference, with a significance level of $P < 0.05$, and an error of estimation of ±0.6% (3.5 ± 0.6%), a total of 3,600 sera were needed. Differences in antibody prevalence with increasing age were evaluated using the chi-square test with continuity correction. Confidence intervals of 95% were calculated according to the binomial exact method. Sample size calculation and statistical analyses were carried out using EPIINFO 6.02 software (CDC, Atlanta, GA).

RESULTS

Of the 3,974 samples tested, 143 (3.6%; 95% CI: 3.1–4.2%) were positive for HSV-2 antibodies. Initially, 31 specimens were classified as equivocal. These samples were retested by a second ELISA as described under Material and Methods. Only four sera gave a repeatedly equivocal result.

Overall rates of HSV-2 positive subjects, according to age and gender, are shown in Table I. There were no significant differences between age groups in the number of subjects with HSV-2 antibodies. The overall prevalence was 70/1922 (3.7%; 95% CI: 2.8–4.6%) in males and 73/2052 (3.6%; 95% CI: 2.8–4.5%) in fe-

males. Comparisons between males and females in each of the strata did not reach statistical significance. Seropositivity rates did not differ among the different geographic regions.

DISCUSSION

Until very recently, tests able to detect type-specific gG2 antibodies have not been adapted for commercial use and their availability was restricted to a few research centres. To our knowledge, this is the first HSV-2 seroepidemiological survey based on a reliable type-specific enzyme immunoassay (recombinant gG2 ELISA), conducted on a representative sample of the Spanish population.

According to the results of this study, only 3.6% of the Spanish population aged 5–59 years exhibits antibodies to HSV-2. This epidemiological situation is dramatically different to that described in the USA, where overall seroprevalences in two large population-based HSV-2 surveys were 16% [Johnson et al., 1989] and 22% [Fleming et al., 1997], whereas in Europe varies from 8% to 33% [Ades et al., 1989; Nahmias et al., 1990; Kjaer et al., 1990, 1993; Cowan et al., 1994; Forsgren et al., 1994]. Most of these European studies have been conducted in selected groups or populations and involved different serological assays, thus making the results difficult to compare. Nevertheless, it is likely that geographical factors are also important, resulting in variations in prevalence among different countries.

Spanish studies describing the prevalence of antibodies to HSV-2 both in selected groups and in the general population are also limited. Seroprevalence rates have varied according to the population studied and the characteristics of the antigen used in different ELISA kits: 10% and 7% in pregnant women and their husbands from Seville (purified gG2) [Nahmias et al., 1990], 9% in pregnant women from Madrid (whole cell lysate antigen) [Vizcaíno et al., 1987], and 11% and 12% in women who were controls in two studies on the association between cervical cancer and different sexually transmitted pathogens (purified gC2) [De Sanjosé et al., 1994]. In a recent study carried out on sera taken from a representative sample of women of childbearing age from Madrid (purified gG2), the overall prevalence of HSV-2 antibodies was 3.5%, thus being very similar to the overall prevalence obtained in the present study [Ory et al., 1997]. Even lower rates (1%) have been recorded in university students (recombinant gG2) [García-Corbeira et al., 1997].

Sexual transmission by intimate genital and orogenital contact and perinatal transmission from mother to baby are the two main sources for HSV-2 shedding in the community [Straus, 1995]. In the present study, however, the prevalence of HSV-2 did not increase about the time of adolescence as a reflection of the initiation of sexual activity. Furthermore, detection of HSV-2 antibodies was not associated with increasing age as would be expected for a sexually transmitted disease and has been described in previous studies carried out in the USA [Johnson et al., 1989; Fleming et al., 1997] and Europe [Ades et al., 1989; Kjaer et al., 1990; Cowan et al., 1994]. It should be noted that subjects in the age range 5-12 years exhibited a similar seroprevalence to the rest of the age groups, suggesting that the HSV-2 virus is not circulating among the general population. It is likely that virus circulation is restricted to certain risk groups in the community as suggested by seroprevalence rates found in STD patients (22% in women and 13% in men) and homosexual men (22%) from Seville (Spain) [Nahmias et al., 1990].

Owing to the prolonged intimate contact between mother and baby during the perinatal period, and the fact that HSV-2 has more opportunities of being transmitted asymptomatically [Mertz et al., 1992; Straus, 1995; Mindel, 1998] and may occur from multiple anatomical sites [Wald et al., 1995], it is not surprising to find that the seroprevalence rate in children (5–12 years age group) did not differ from that obtained in females of childbearing potential. As suggested by the data obtained in this study it seems that in Spain perinatal viral shedding represents the moment of maximal HSV-2 acquisition in the general population. Once it is acquired, prevalence is maintained in a similar proportion through the different age groups.

In conclusion, the present study suggests that HSV-2 seroprevalence rates in the general Spanish population are among the lowest in Western Europe. Perinatal virus shedding from mother to baby seems to be a more important source of HSV-2 seroconversion than sexual transmission.

ACKNOWLEDGMENTS

We thank Dr. J.A. Gómez-Gerique, Dr. J.A. Gutierrez-Fuentes, Dr. M.T. Montoya and Dr. A. Porres and all the DRECE study group for supplying the samples used in the present study and M.J. Salvador for her technical assistance.

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